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13. ABSTRACT (Maximum 200 words)

The influence of diesel fuel on benthic food webs in coastal saltmarshes were examined in a series of microcosm experiments. Diesel contamination resulted in high mortality to all copepods except *Cletocamptus deitersi*, while nematode abundance increased. Blooms of benthic microalgae that developed in contaminated sediment were primarily a response to reduced copepod grazing, and secondarily a consequence of enhanced NH_4^+ flux from sediments. Microalgal grazing by nematodes and copepods was enhanced in contaminated sediments, and was a consequence of *C. deitersi* competition with copepods other than *C. deitersi*. Predation by fish was reduced by 60% at PAH levels higher than $\geq$ 78 ppm; at $\geq$ 300 ppm, no feeding occurred. A comparison of benthic communities from a relatively pristine and chronically contaminated marsh systems revealed that the general response to diesel contamination was similar (general mortality to crustaceans, increased nematode abundance, and microalgal blooms), but the effect was more pronounced at the relatively pristine site. Collectively, our research has yielded an understanding of how and why benthic communities in salt marshes respond to diesel: benthic invertebrate community structure, grazing on benthic microalgae, and nitrogen biogeochemistry are altered and ultimately lead to algal blooms and changes in competitive interactions among benthic invertebrates.

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FINAL REPORT

GRANT #: N00014-93-1-0975

PRINCIPAL INVESTIGATOR: Kevin R. Carman

INSTITUTION: Louisiana State University

GRANT TITLE(S): (1)An experimental investigation of the influence of diesel fuel on the food webs of two sedimentary communities/ (2) Direct and indirect effects of diesel fuel on microphytobenthos and meiofauna in saltmarsh sediments

AWARD PERIOD: 1 July, 1994 - 30 Oct., 1998

OBJECTIVES: (1) To determine the influence of diesel fuel on functional and structural attributes of salt-marsh, benthic food webs in two habitats, one that has been chronically exposed to petroleum hydrocarbons for decades (Louisiana - LA), and another that is relatively pristine (Mississippi - MS).

(2)To determine if(i) algal blooms in contaminated sediments are the result of direct or indirect stimulation from diesel or from a reduction in meiofaunal grazing; (ii) mortality to copepods in contaminated sediments is from direct toxicity from diesel, anoxic conditions, or a combination of the two; and (iii) *Cletocamptus deitersi* (a meiofaunal copepod) thrives in contaminated sediments because of reduced competition from other species, or exploitation of altered algal resources.

APPROACH: (1)Microcosm experiments were used to examine the influence of diesel fuel on the microbial and meiofaunal components of sediment food webs in Louisiana (LA-contaminated site) and Mississippi (MS-pristine site). Microcosms were exposed to sediment that had been contaminated with diesel fuel over concentrations ranging from 0 to 80 ppm PAH (polycyclic aromatic hydrocarbons). The impact of diesel was determined from a variety of measurements on the microbial and meiofaunal components of the communities, including microalgal and bacterial abundance, activity, and physiological condition; bacterial metabolism of PAH; meiofaunal grazing on microalgae; and meiofaunal community composition. The impact of diesel-contaminated sediments on trophic interactions between juvenile fish and meiofauna was also examined in separate laboratory experiments. Experiments were designed to determine how fish respond to sediment contaminated with diesel.

(2)Microcosms experiments were performed to determine the effects of diesel-contaminated sediment on microalgal-meiofaunal interactions. Natural sediments were defaunated, then manipulated to control meiofaunal abundance and species composition, as well as diesel contamination. Treatments were compared to determine if reduced grazing or altered nutrient availability in diesel-contaminated sediments lead to algal blooms. Toxicity tests were used to determine interspecific differences in sensitivity to diesel and oxygen depletion. The influence of diesel on meiofaunal competitive interactions (grazing rates) was measured in communities manipulated to mimic those that occur in diesel-contaminated sediments. Microcosm experiments were performed to determine if enhanced N availability in diesel-contaminated sediments was from decaying carcasses of meiofauna, or altered sedimentary biogeochemical processes. HPLC analysis was employed to determine changes in sedimentary microphytobenthos, and how those changes relate to copepod feeding.

ACCOMPLISHMENTS: (1) In 1994, the influence of diesel was examined in Louisiana (LA) saltmarsh sediments. At the highest level of contamination (ca. 80 ppm PAH final concentration) the benthic food web was impacted in several ways relative to controls and other treatments: (i) Microalgal biomass and activity increased by approximately 10x; (ii) meiofaunal community

structure was significantly altered. With the exception of *Cletocamptus deitersi*, whose abundance increased in the most contaminated treatment, essentially all copepods were eliminated; (iii) Grazing rates of nematodes and *C. deitersi* increased; (iv) Although traditional measures of bacterial abundance and activity indicated no effect of diesel, a significant (and dose-dependent) increase in bacterial PAH degradation was detected.

The effect of diesel-contaminated sediment on feeding by the darter goby (*Gobionellus boleosoma*) was tested under sediment contamination levels ranging from 0 to 625 ppm. A significant (60%) reduction in prey consumed occurred at PAH levels higher than 78 ppm. At > 300 ppm, all feeding was inhibited.

The 1995 microcosm experiment compared the influence of diesel-contaminated sediment on the meiofaunal/ microbial food webs of chronically contaminated (LA) and relatively pristine (MS) sites. In the 1995 experiment, responses to diesel contamination were fewer and of a lesser magnitude than those detected in 1994. Nevertheless, the major responses detected in the 1994 experiment were evident again in 1995, giving us confidence in the generality of our conclusions. However, ambient algal biomass was higher in 1995 than in 1994, and the increase in algal biomass in 1995 High treatments was not as pronounced as was observed in 1994, which may have been a consequence of reduced meiofaunal grazing in 1995. (iii) Copepod grazing was significantly suppressed in Medium and High treatments. However, field copepod abundances were lower in 1995 than in 1994, and thus their total grazing pressure on benthic microalgae was lower and algal biomass was not as tightly controlled by grazing as it was in 1994. Thus, negative effects of diesel on copepod grazing (which were relatively low because of high bacterial metabolism of hydrocarbons) did not have the dramatic impact on algal biomass that it did in 1994. (iv) *Cletocamptus deitersi* became disproportionately abundant in High treatments, but the response less than in 1994, probably because of reduced toxic effects from diesel resulting from rapid metabolism of diesel by benthic bacteria.

The response to diesel in MS and LA sediments also displayed important differences, which suggest that the MS community was more sensitive to hydrocarbons: (i) Total copepod abundance decreased in Medium and High treatments in MS, but not in LA microcosms. (ii) Copepod nauplii increased in LA, but decreased in MS microcosms. (iii) The nematode:copepod ratio, an index of pollution, was strongly affected in MS but not LA microcosms.

(2) The effects of altered grazing and nutrient availability were examined in two microcosm experiments. In the first, we determined if diesel contamination influences NH_4^+ flux from sediments. Results indicate an enhanced flux of NH_4^+ from contaminated sediments. NH_4^+ concentrations remained very low in contaminated sediments incubated in the light, because microalgae consumed it as fast as it was produced. Addition of NH_4^+ to uncontaminated sediments did not stimulate algal growth, however, indicating that N availability was not the primary factor limiting algal growth.

In a second experiment, we examined the hypothesis that reduced grazing led to the formation of algal blooms. Meiofaunal abundance was manipulated to remove copepods and other larger grazers from sediment. Algal responses to this manipulation of grazing were compared to those that occurred in diesel-contaminated sediments. Both removal of grazers and contamination with diesel produced a 2-fold increase in algal biomass over a 4 days. Microscopic and HPLC analyses indicate that the increased algal biomass was almost entirely from growth of diatoms.

Experiments were also conducted to distinguish between the effects of hypoxia vs direct toxicity of diesel on meiofaunal copepods. In all species examined, we observed that hypoxia greatly increased the toxicity of diesel-contaminated sediment. *Cletocamptus deitersi*, a species that seems to be resistant to diesel toxicity, was the least sensitive of the species tested.

In previous microcosm experiments using natural communities, we observed that nematodes and *C. deitersi* thrive and exhibit enhanced grazing rates on microphytobenthos, leading to the hypothesis that *C. deitersi* and nematodes

benefit from a release from competition for food when other copepods are killed. This hypothesis was tested by experimentally manipulating *C. deitersi* and nematode abundances to simulate conditions that occur in diesel-contaminated sediment. Grazing by *C. deitersi* and nematodes increased in the absence of copepod competitors. Among nematodes, enhanced grazing on algae was limited to a diatom feeder, *Ptycholaimellus* sp. Our evidence indicates that increased grazing by *C. deitersi* in contaminated sediments is partially from reduced competition, and partially a response to the enhanced algal biomass in contaminated sediments.

Our research indicates that algal blooms in diesel-contaminated sediments are a consequence of reduced grazing (from meiofaunal mortality) over short periods (5-14 days), followed by a second growth phase that is fueled by enhanced N fluxes. We performed two experiments to examine the source of this excess nitrogen. The first examined the possibility that N comes from decaying carcasses of animals killed via diesel toxicity. Results indicate that nitrogen released from decaying carcasses occurs over very short periods (3-4 days), well before algae become N-limited. Thus, excess N that fuels growth of algae must come from other N pools in the sediment.

Observations above led to an experiment (performed last fall) in which we examined in more detail the biogeochemistry of N dynamics in diesel-contaminated sediment. We are in the final stages of analyzing samples from this experiment. We are examining the hypothesis that altered microbial activity in diesel-contaminated sediment may result in increased metabolism of refractory marsh detritus, which contains geoplymers that may have "complexed" DIN; this could result in the release of organic and inorganic nitrogen. We are measuring total hydrolyzable and dissolved free amino acids (THAA) in experimental sediments from diesel-contaminated and control sediments, as well as N and P dynamics in inorganic pools (PO4, NH4, NO3, and NO2), and bulk sediment nitrogen. Analysis of inorganic pools has been completed, and analysis of amino acids and bulk sediment is underway. Preliminary analysis indicates that diesel contamination dramatically enhances the NH_4 (as previously documented) and PO_4 flux from contaminated sediments relative to controls. Evidence of enhanced PO_4 flux is new, and may contribute to the formation of microphytobenthos blooms in contaminated sediments. When amino-acid and bulk-sediment analyses are completed, we should have a much clearer picture of the origin of excess nitrogen that fluxes from contaminated sediments.

CONCLUSIONS: Results provide several insights into the influence of diesel contaminants on benthic food webs. Benthic microalgae constitute the base of this food web, and thus their dramatic increase in response to contaminants is ecologically significant. The consistency of results from 1994 and 1995 experiments indicates the robustness of our observations of reduced copepod grazing and enhanced algal biomass in the presence of high levels of diesel contamination. While the broad responses of the two communities were similar, differences in the response of the meiofaunal communities are consistent with the hypothesis that the MS community is more sensitive to contaminants. Microalgal biomass in sediments is controlled primarily by meiofaunal grazing pressure. Mortality to meiofaunal grazers (which occurs as a consequence of the interactive effects of hypoxia and diesel toxicity) releases algae from grazing pressure. The algae grow until they become N-limited. Enhanced NH_4^+ production in diesel-contaminated sediments then allows algal blooms to develop further. Thus, our experiments have allowed us to identify the general process by which blooms occur in diesel-contaminated sediments.

SIGNIFICANCE: Collectively, our research has yielded an understanding of how and why benthic communities in salt marshes respond to diesel. Diesel contamination dramatically alters benthic invertebrate community structure, grazing on microphytobenthos, and nitrogen biogeochemistry. These cascading effects lead to algal blooms and fundamental changes in competitive

interactions among benthic invertebrates.

AWARD INFORMATION: K. Carman was awarded tenure in 1996; T. Bianchi was awarded tenure in 1998. K. Carman received the College of Basic Sciences "Faculty Research Award" in 1996.

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Bianchi, T.S. & K.R. Carman. The effects of PAH contamination on the availability of nitrogen to microphytobenthos in marsh sediments Org. Geochem. (In prep).

Abstracts

1998 *Benthic Ecology Meeting* (4)
Invited seminars given by Carman at Florida State University, Old Dominion Univ., Inst. for Marine Sciences (UNC Chapel Hill marine lab), Univ. North Carolina Chapel Hill, Univ. South Carolina

1997 *American Society for Limnology & Oceanography* (3)

1996 *Ocean Sciences Meeting; Benthic Ecology Meeting*

1995 *Ninth International Meiofauna Conference; Benthic Ecology Meeting* (3)

1994 *Society for Environmental Toxicology and Chemistry*